2. The Smooth Muscle Cell. The Pivot in Atherosclerosis

"Whereas the precise nature of the initiating event for atherosclerosis is not known, it is clear that the failure of the smooth muscle cell to maintain its normal differentiated phenotypic state becomes a key contributing factor in the progression of atherosclerotic disease."
GK Owens, [17]

The Fibroproliferative Response

Atherosclerosis is generally considered a fibroproliferative (FP) disease because atherosclerotic plaques contain large amounts of fibrous tissue [2]. This FP response is believed to be a defensive, protective, physiologic response to injury, designed to wall off, contain, enclose, or sequester the IA, and then to assist in resolution of the injury [2]. However there is no evidence to show that the FP response ever functions in a defensive or protective fashion.

Continued plaque growth suggests the resident intimal SMCs and any SMCs migrating from the media are being stimulated to produce more and more fibrous tissue, but this continued growth does not prove that the FP response is a physiologic defensive response to injury. If the growth and proliferation of fibrous tissue were a defensive response, then why isn't the disease process halted in its very earliest stages, and why does the FP response progress above and beyond that required to repair a small area of injury [2,12]?

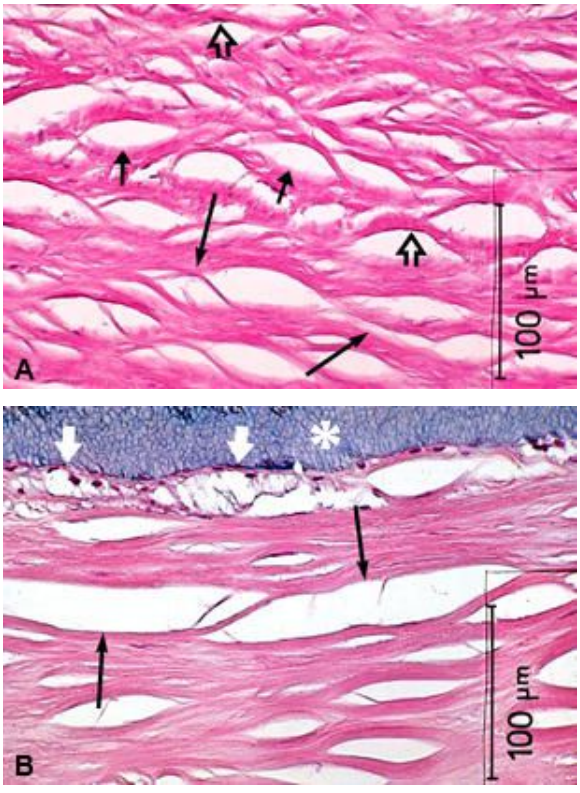
Plaque tissue is produced primarily by intimal SMCs, not by fibroblasts, the usual cell type normally involved in wound repair: it therefore differs from the normal fibrotic response to injury [12,27]. The FP response may not be a defensive mechanism gone awry [2], but a pathologic component of the disease process from the very beginning. There is no reason to think the FP response is physiologic in any way because all atherosclerotic plaque tissue represents diseased tissue, regardless of the circumstances under which it formed.

This chapter presents evidence supporting the view that the intimal SMC is specifically targeted, its intracellular functions altered by an IA, to produce a specific type of pathologic fibrous tissue. We hypothesize that inherent SMC functions are altered to such an extent that the SMC becomes a participant, mediator, and perpetrator of the disease process, as illustrated in the following examples.

Atherosclerotic Fibrous Tissue

Typical plaque fibrous tissue, illustrated in Figures 3A and 3B, is found throughout atherosclerotic plaques. The tissue architecture consists of a meshwork of collagen bundles embedded in ECM, with many lacunar-like spaces containing SMCs staining positive with SMC actin antibody (Figure 3C). The collagen and other components of the ECM are believed to be produced by these SMCs (2,4,12,27), forming an unusual and distinctive type of fibrous tissue. The SMC nucleus is flattened against one side, suggesting compression or increased pressure within the lacunar space (Figures 3A, 3B). These SMCs appear to be identical to those shown in Figure 1, except that these are larger and enclosed in a uniform fibrous meshwork. Other investigators have observed these flattened, attenuated SMCs [28,29].

The fibrous tissue shown in Figures 3A, 3B is relatively acellular, similar to the asymmetric intimal thickening shown in Figure 1, representing a further developmental stage due to the large amount of collagen and ECM. The SMCs within these lacunar spaces appear to be filled with clear material, presumably lipid, that is compressing the nucleus. The type of lipid has not been identified, but it appears to be in a different form than that found in macrophage foam cells (Figure 3B). Lipid droplets are not obvious, so the SMC may metabolize ingested lipid differently from monocyte-derived macrophages [30,31]. The absence of a typical foam cell configuration may be due to a lack of esterification of lipid by the SMC. Other investigators, using electron microscopy, have noted that SMC do contain lipid droplets, showing that at least some of the lipid ingested by SMC is esterified, but seldom to the same extent as monocyte-derived macrophages [17,31].



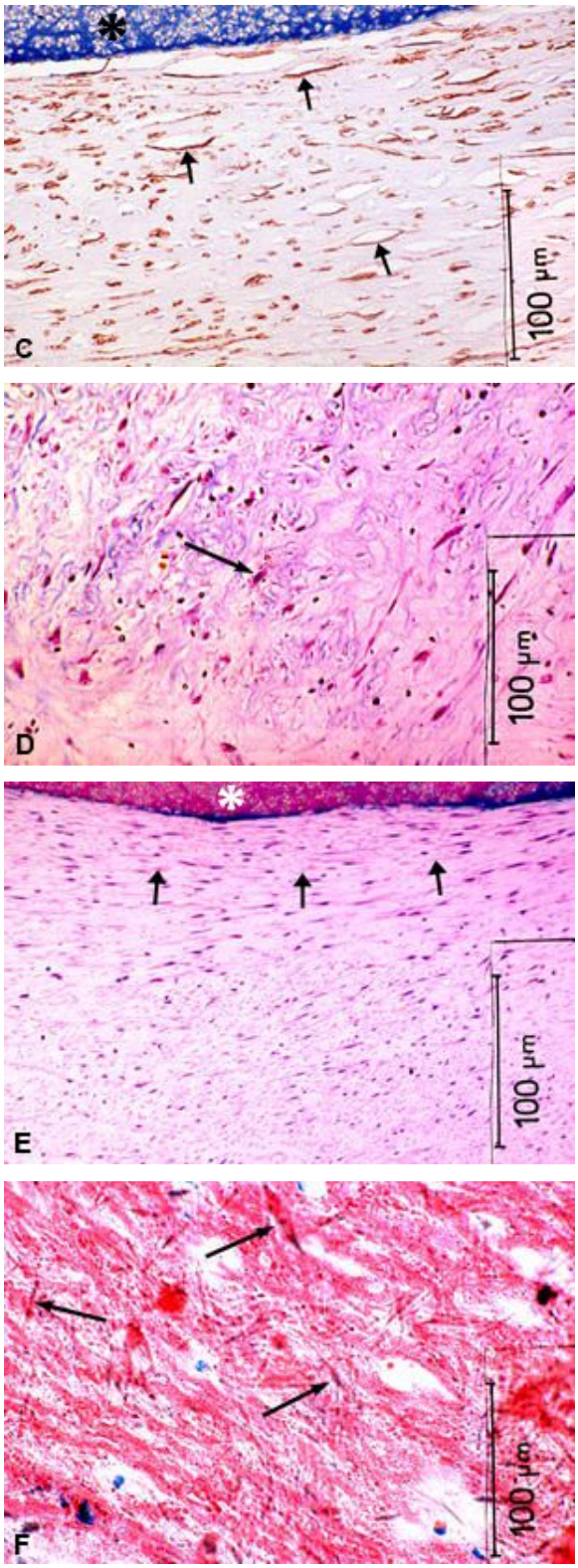


Figure 3: **A**, Typical fibrous plaque in a 65-year-old white male. Note the spindle-shaped, lipid-laden, SMCs (small arrows). These cells vary greatly in size, and some appear to be coalescing with neighboring cells (long arrows). The SMC nucleus is flattened along one side of the lacunar spaces (open

arrows). There are relatively few cells present, but a large amount of fibrous tissue. H & E stain. **B**, High-power view of the subendothelial area in the same section as **A**. There are foam cells (white arrows) and other lipid- containing cells just beneath the endothelium. Two very large, lipid-filled spaces, apparently formed by the joining of adjacent, but dead, SMCs are present (black arrows). Asterisk = Lumen. H & E stain. **C**, A small fibrous plaque in a 50-year-old female, stained with SMC actin, showing the lipid-laden cells (arrows) within the lacunar spaces are, in fact, SMCs. Asterisk = Lumen. **D**, Intimal hyperplasia in a 75-year-old white female who received PTCA at this site 9 months earlier. Note the hypercellularity, the disorganization of these cells, the presence of stellate cells (arrow), and the absence of any lipid-laden SMCs. H & E stain. **E**, Intimal hypercellularity in a 6-month-old vein bypass graft in a 53-year-old white male. The SMCs are oriented parallel to the endothelial surface, and none appear to be lipid-laden. Note the hypercellularity near the endothelial surface (arrows). Asterisk = Lumen. H & E stain. **F**, Oil Red O stain of a fibrous plaque showing the fibrous tissue diffusely infiltrated with lipid droplets, giving the fibers a granular appearance. Note the scattered cholesterol crystals (arrows).

Other Types of Vascular Fibrous Tissue

Contrast the relatively acellular tissue in Figures 3A and 3B, with the hypercellular response seen following percutaneous transluminal coronary angioplasty (PTCA), (Figure 3D), or a recently placed saphenous vein bypass graft, Figure 3E. These structural differences plus the absence of lipid-laden SMCs in Figures 3D, 3E, indicate that these tissues are not, or at least not yet, atherosclerotic. Presumably the FP response following PTCA is related to physical injury produced by the balloon, and the thickening of the vein graft wall is a physiologic, hyperplastic response to increased hemodynamic stress within the vein graft lumen.

These different cellular and structural responses to different types of vascular injury suggest different IAs stimulate the SMC to produce different FP responses, illustrating the pleuri-potential nature of the SMC [17]. The monoclonality of the SMC, proposed by Benditt [32], suggests the SMC is altered in a very specific way to produce a very specific type of fibrous tissue [12]. Recent evidence shows different types of SMCs in plaques, but the histologic picture is the same in all plaques [12] and no pathognomonic changes distinguish one IA from another.

The Smooth Muscle Cell

The SMCs found in plaques have an altered phenotype compared with normal SMCs, but what controls these phenotypic changes and whether they are the cause or the consequence of active atherosclerosis is unknown [17]. These SMC show altered lipid metabolism, altered growth factor production, altered ECM production, smaller size, fewer intercellular junctions, and the presence of fatty vacuoles [12,32]. In addition there is a decrease in myofilaments and various proteins, with an increase in golgi and rough endoplasmic reticulum [17]. Although this phenotypic change is often considered a physiologic response to an injurious stimulus, the possibility that this change is actually a pathologic alteration in SMC function and a key factor in the development of plaque lesions cannot be excluded [17].

The structural meshwork of the tissue shown in Figures 3A and 3B suggests these lacunar spaces may be created by the ingestion of lipid by the enclosed SMC [33]. The flattening of the SMC nucleus and the variation in size of lacunar spaces suggest over-ingestion and/ or unregulated uptake of lipid by the SMC is greater or more advanced in some SMCs than in others. The inability to regulate the uptake of lipid is one of the features of monocyte-derived macrophages found in plaque tissue [31]. The lipid-laden SMC in Figures 3A, 3B may be SMC macrophages that have also lost that regulatory ability [1,21,30,31]. The loss of lipid regulatory capacity is believed due primarily to the presence of scavenger receptors on the SMC [34].

SMCs are reported to transform into macrophages and to take on the appearance of foam cells, but they are not as efficient as monocyte-derived macrophages in the uptake of lipid, possibly because SMCs do not express scavenger receptors to the same degree as do monocytes [2,31]. Further, some of the larger lipid-laden SMCs appear to be fusing with adjacent lipid-laden SMCs resulting in the formation of small lipid lakes, suggesting active and continuing accumulation of lipid (Figure 3B) [10,35].

If, in fact, these are SMC macrophages, why have they formed in this location, what is their purpose, and why are they ingesting lipid? The general purpose of the macrophage is to neutralize and remove any IA and/or harmful or toxic compounds and to participate in the removal of dead and injured tissue [36,37]. Since this is atherosclerotic plaque tissue, we can assume that three of these factors, the IA, cytotoxic compounds, and injured tissue are present within a plaque.

Figure 3F shows plaque fibrous tissue is heavily infiltrated with lipid, is extra-cellular [33] and is presumably the source of lipid ingested by the SMC. The metabolism or oxidation of this retained lipid, an expected consequence of lipoprotein trapping [19], results in the formation of cytotoxic compounds such as oxidized LDL [35,38]. These compounds may produce the harmful stimulus necessary to transform the SMCs into macrophages [31]. We can assume that one of the reasons these SMC are taking up lipid is because it is a toxic substance or compound [30,31].

However, these SMCs do not seem to neutralize or remove the lipid or toxic compounds, but simply ingest more and more of them, resulting in fibrous tissue laced with lipid-laden SMCs of different size (Figures 3A, 3B). Nor is there any evidence that the IA and/or the injurious process have been slowed, halted, or the injury resolved by the activity of the SMC macrophages. The inability to remove lipid from the artery wall, for instance, by reverse transport, may be related to a decrease in mobility of SMCs related to the thick fibrous network. Or the SMC, like the monocyte-derived macrophages, may be partially disabled and have decreased mobility through the excess intake of lipid [30]. Is it possible that some or all of these SMC functions are pathologic components not physiologic defenses, of the disease process? Whatever the mechanism, the SMC appears to be a key player, their normal functions targeted and subverted by the IA, serving to mediate the subsequent development of atherosclerotic disease.

Degeneration and Necrosis

The fibrous tissue shown in Figures 3A and 3B appears to be fatally flawed. All such tissue, given sufficient time, may degenerate and undergo necrosis, forming a lipid-laden necrotic core or atheroma. The SMCs die as result of toxic chemical agents such as oxidized LDL, from the over-ingestion of lipids, from hypoxia or from apoptosis [25,37,39–41]. The uptake of oxidized LDL by SMC macrophages may promote apoptosis [39]. The death of the SMC leads to discharge of the ingested lipids and other cellular elements into the extracellular space, and to the degeneration of non-viable fibrous tissue. Degeneration and necrosis of the lipid-laden fibrous tissue lead to a further increase in the extracellular lipid content, and eventually to a lipid-rich necrotic core.

Figure 4 illustrates degeneration and destruction of tissue surrounding the necrotic core. Figure 4A shows a relatively small plaque with a necrotic core and an overlying fibrous cap. A fibrous cap is generally considered to be a protective layer of fibrous

tissue separating the core from the lumen, formed primarily to contain and prevent communication between the lumen and the necrotic core [4].

The necrotic core in Figure 4A appears to be expanding toward the shoulders of the plaque, illustrating the tendency for the plaque and the necrotic core to grow circumferentially, as in Figure 2A. Erosion through the fibrous cap will probably occur first at the lower shoulder of the plaque where the fibrous cap is thinnest. The histologic structure of the fibrous tissue in this fibrous cap is the same as that illustrated in Figures 3A, 3B. This fibrous cap, already diseased and weakened by dysfunctional SMCs, is destined for eventual destruction because the SMC in the cap will eventually die, the cap will degenerate, and the plaque will rupture. In other words, the IA appears to affect and destroy the SMCs in the fibrous cap and elsewhere, by creating a toxic necrotic core that destroys surrounding tissue, including the fibrous cap. Thus, the fibrous cap has become a pathologic component of the disease process, not a protective structure designed to enclose the necrotic core.

In Figure 4B, the fibrous cap adjacent to the lumen in the upper part of the photo separates the lumen from the deeper necrotic core. The tissue between the necrotic core and the fibrous cap is in the process of degeneration and destruction and will soon be part of the core, presumably as a result of the toxic substances within the necrotic core, the growth and expansion of the necrotic core, and the resulting death of SMCs [25,40–42].

The expanding destruction of tissue surrounding the necrotic core is vividly apparent in Figure 4C. These photos illustrate gradations and zones of tissue damage and destruction around the necrotic core from the center of the core outward. They also show that although there is initial proliferation of fibrous tissue in atherosclerotic disease [31], the subsequent destruction of this same tissue [39] means the IA and subsequent atherosclerotic disease are basically and ultimately destructive in nature [43].

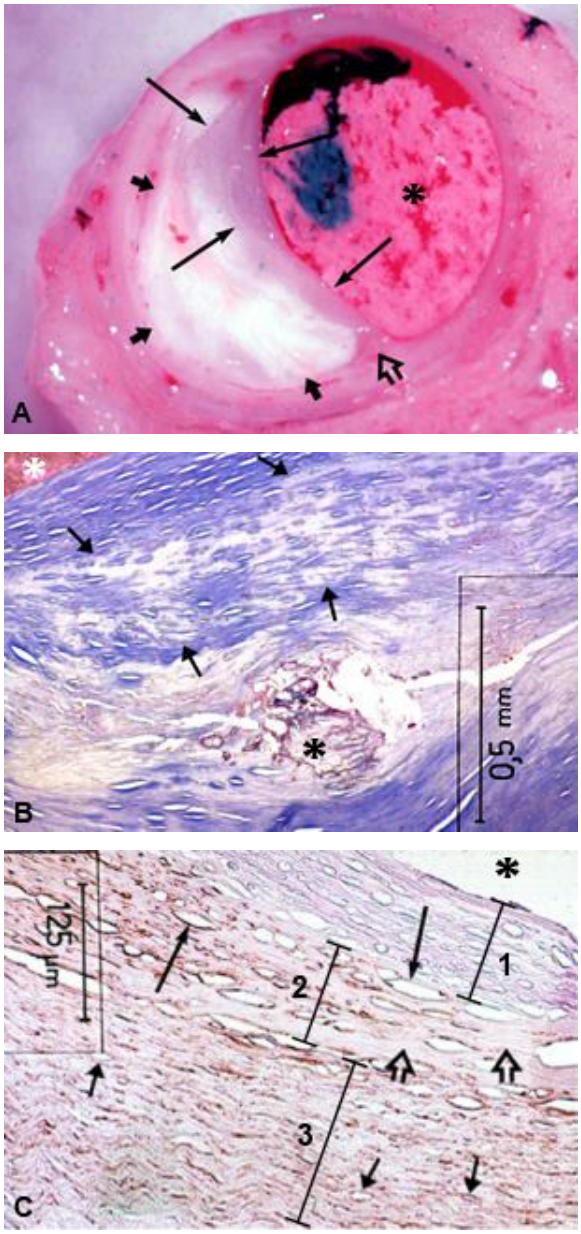


Figure 4: **A**, Crescent-shaped atherosclerotic plaque in the proximal LAD coronary artery of a 53-year-old white male. Red and blue injection mass has mixed in the lumen (asterisk). Note the major part of the plaque is composed of whitish material (fat arrows), and microscopic examination shows this white material to be the necrotic core. The fibrous cap is made up of what appears to be clear or transparent tissue (thin arrows) and varies in thickness from one side of the plaque to the other. The fibrous cap is quite thin at the plaque shoulder near the bottom of the photograph (open arrow). **B**, High-power view of the plaque

shown in **A**. The necrotic core is identified by a black asterisk, and the arterial lumen by a white asterisk. The fibrous cap has structure similar to that shown in Figures 3A and 3B, is adjacent to the lumen. The fibrous tissue (arrows) between the lumen and the necrotic core has undergone partial degeneration and necrosis. MSB stain. **C**, SMC actin stain of a fibrous plaque surrounding a necrotic core (asterisk) from another patient. The tissue adjacent to the necrotic core has undergone degeneration with loss of SMCs, but the basic fibrous structure remains (Bracket 1). The next layer outward from the necrotic core (Bracket 2), shows the SMCs are still apparently viable and stain appropriately with the SMC actin stain, but many are lipid-laden (long arrows), of different size, with several small lipid lakes and with early degeneration of tissue at the right side of the photograph (open arrows). Beyond this second layer is a layer of cellular fibrous tissue (Bracket 3) composed of viable-appearing SMCs, and only occasional small lipid-laden SMCs (short arrows).

In Review

Atherosclerotic fibrous tissue is diseased, pathologic tissue produced by SMC's that have been targeted and affected and whose intracellular functions have been altered by the IA to produce a specific, pathognomonic, type of fibrous tissue. Plaque fibrous tissue is laced with lipid laden SMCs, probably SMC macrophages, whose normal function has been subverted by the IA, leading to the eventual death of the SMC and degeneration of the fibrous tissue. The SMC is the key cell affected by the IA, and it mediates the onset and progression of atherosclerotic disease. The fibrous cap is not a specialized protective structure but is a typical atherosclerotic fibrous tissue formed by phenotypically altered SMCs. It only appears to be a cap because the necrotic core tends to form first in the central part of the plaque. Atherosclerosis, in the final analysis, is basically and primarily a destructive disease process.

Unanswered Questions

How does the IA enter the wall? Why is the SMC targeted and intracellular functions altered? Why does the IA first stimulate the proliferation of fibrous tissue, then proceed to destroy this same tissue? Is this the nature of the disease process, and if so, what purpose is served by destroying this tissue? What is the mechanism of growth and expansion of the necrotic core? Are the SMCs responsible for the FP response the same cells that transform into macrophages and ingest lipid, as Owens suggests [17]? Can the SMCs that migrate from the media undergo a phenotypic change from producing contractile fibers to synthesizing fibrous tissue, then subsequently transform or be transformed into a SMC macrophage? If so, does this confer a survival advantage [17], or are these migrating SMCs subverted in the same manner as resident SMCs, becoming part of the disease process?

Can one type of SMC perform all these various functions, or are there various subsets of SMCs that transform along certain cell lines, each performing a different function [44]? Is failure to regulate SMC differentiation [17] due to the effects of the IA? How can a cell that is partially disabled, say with scavenger receptors, continue to manufacture and secrete sophisticated and complicated metabolic compounds, such as growth factors and pro teolytic enzymes? Whatever the answers to these questions, clearly the SMC plays a major role in the progression and development of atherosclerotic lesions.